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# Influence of Ovaprim and Pituitary Gland on The Reproductive Indices and Growth of *Clarias garipienus* (Burchell, 1822) Broodstock Reared in An Indoor and Outdoor Pond

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**Abstract** Ovaprim and pituitary gland from *C. gariepinus* was used to ascertain the reproductive indices and growth value of the hatchlings (F1) of *C. gariepinus* at the Fisheries and Research Institute Baga. Broodstocks mean body weight males (1200 g) and females (800 g) was collected from Lake Chad and transported to the institute hatchery complex. It was injected with ovaprim and pituitary gland. The fecundity, sperm volume and weight of the testes were estimated after the latency period. While the hatchlings were reared for eight weeks in a complete randomized design setup in an indoor and outdoor facility to evaluate the weight and length increase. The result revealed significant difference ($P$>0.05) in the fecundity of the females, ovaprim had 10,500 while pituitary gland had 7,000. There was significant difference ($P$>0.05) in % fertility and hatchability of the eggs. However, the male injected with ovaprim had higher milt volume (2.2 mL). Subsequently, there was no significant difference ($P$>0.05) in weight and length parameters across the treatments for the period of the research, the mean weight gains of fry reared indoor induced with ovaprim was 8.77 g while pituitary gland extract was 8.13 g. Meanwhile, the fingerlings reared four weeks in an outdoor pond had a significant growth, the fingerlings induced with ovaprim had 14.35 g in weight gain while pituitary gland was 14.42 g. There was no significant ($P$>0.05) difference between the two hormones on fingerling production. There was no significant difference ($P$>0.05) between increase in length of fry reared indoors and outdoors for four weeks. This study proved that pituitary can effectively be used to induce Catfish breeding, when considering its availability and high cost of purchasing ovaprim.

**Keywords** Pituitary gland; Ovaprim; Reproductive indices; Growth and *C. gariepinus*

## Background

Fish are the most resourceful of animals in terms of reproduction. Impairment of reproduction in captivity can be partial or total (Taranger et al., 2009). However, in some species of fish, the gonads may mature at same time; while in another fish of same age the gonad may mature and even produce viable gametes while the other do not (Beirao et al., 2019). In many species, these may tarry after wild fish of the same age have spawned repeatedly. Fish farmers need fry or seed in very large numbers. Recently, with low catch from the wild, there is a need to increase production through aquaculture (Oguntuase and Adebayo, 2014; Naylor et al., 2000). The better the reproductive physiology of fishes is understood, the more chances of success with induced breeding (Ochokwu et al., 2015).

However, Fraser (2008) reported that the challenge is to balance the cost of providing a sufficiently “natural” captive environment against the effectiveness of intervening with hormones. In such case many species will spawn without synthetic hormones with environmental manipulation A complementary approach, environmental manipulation and more attention to broodstock require diet, water quality, stocking and holding conditions are combined to enhance the outcome of hormone techniques, can also yield productively (FAO, 2007).

Including development of endocrine gland techniques, interest has recently increased in using environmental optimization to bring cultured fish to spawn (Migaud et al., 2013). Bayne (2017) reported that culture conditions differ from those in the natural environment and are often not optimal for the final stages of sexual development, maturation and the production of high-quality gametes. Meanwhile, providing optimal conditions for broodstock

reproduction can reduce the need for pharmacological intervention. In species such as *Clarias gariepinus,* where the environmental regulation of sexual development is apprehended, culturists uphold several stocks under environmental regimes that allow sequential spawning throughout the year (Ochokwu et al., 2015). However, more research on the environmental regulation of reproductive development in tropical species like *(Clarias)* will surely enhance development of technologies for all year round seed supply in Lake Chad region of Northern Nigeria (Gupta et al., 2004).

The demand for catfish fingerlings is very high all over Nigeria but the major problem being experienced by hatchery operators in the dry northern zone of Nigeria is the seasonality in the production of Catfish fingerlings (Miller and Atanda, 2010), the quality of the water, pH, High rate of mortality during the first two weeks after breeding, availability and accessibility of the broodstocks, viable and matured gametes (Migaud et al., 2013). Catfish are known to spawn/breed naturally during the rainy season both in captivity and in the wild (Akankali et al., 2011). The length of the rainy season in various parts of the country, determines the length of the breeding period in those areas (Cochrane et al., 2009). This breeding habit most adversely affects the northern part of the country where the rainy season is limited to only 4-5 months in a year.

So far there have been little studies on the efficacy and effectiveness of these two hormones in the arid zone (Northern Nigeria). Although capture fisheries activities is predominant around Lake Chad Basin, yet this study shall further enlighten prospective fish culturist, towards catfish fingerling production in a more economical techniques and creating possibilities of producing fingerlings throughout the year, by enhancing proper timing in fish seed production in the zone to satisfy the quest of those that have embraced aquaculture. Breeding/fingerling production has been limited to 4-5 months of the rainy season and collection of catfish fingerlings from the wild (open water) has been source of fingerlings and this has not been adequate in term of quantity for aquaculture production (Ponzoni and Nguyen, 2007), leading to importation of fingerlings from the southern regions of Nigeria with losses of a large number due to transportation stress. Hence research of this nature shall solve some of the limitations in arid zone aquaculture. More so, Barange et al. (2018) reported that the resultant reduction in the Lake Chad surface water, water depth and near absence of open water for so long a period might serve as opportunity for practicing flood plain pond culture reducing the stress of fishermen trekking longer distances before reaching the receding lake water bodies. The Lake Chad basin is a very prominent livestock and agricultural area and most investment in these activities depends on the neighboring countries (Niger, Chad, Nigeria and Cameroun Republics) (Zieba et al., 2017), this could burst the increase in fish farming, fish demand and fish marketing activities due to high population and different ethnicity within the region (Zieba et al., 2017). The general objective of this work is to assess the efficacy of ovaprim and pituitary gland extract in Induced reproduction of *Clarias gariepinus* fry/fingerlings in the arid zone of Northern Nigeria. To evaluate the growth of the F1, and subsequently point out the reason while fish farmers in the zone should use ovaprim/pituitary in fish breeding to meet up with the demand for fish.

## 1 Materials and Methods

The experiment was done in the Hatchery Unit of Federal College of Freshwater Fisheries Technology (FCFFT), Baga, Borno State, Nigeria. FCFFT is situated in Kukawa Local Government Area of Borno State, which falls within the Lake Chad Basin. Baga lies on latitude 12$^{o}$-55'N and longitude 13$^{o}$-35'E and has population of about 105,588 people (Biu et al., 2020). The major occupation of people is farming and fishing. The "Baga" fish trading is known all over the country and forms a great item of international trade between Borno and other States. The dominant ethnic group is Kanuri, however, a sizeable number of Hausa, Fulani, Igbo, Yoruba and international tribes from Chad, Niger, Cameroon and other African countries co-exist peacefully by the lake. The peak of the annual rainfall commence in August and end in October. Average water temperature during peaks of breeding season ranges from 22℃ ~ 32℃ and as low as 14℃ in the dry season (November to December) (Sule and Raji, 2003).

### 1.1 Collection and acclimatization of experimental fish

Twenty (20) broodstocks of *C. gariepinus* average weight for males 1.2 kg and females 800 g in a ratio of 12:8 were collected from fisher men at the landing site of Lake Chad and transported to Federal College of Fresh Water Fisheries Technology, Baga in a plastic troughs of 50 cm x 30 cm deep. The physico-chemical parameter of the Lake Chad water was: pH-7.0, water temperature 28℃, Dissolve 0xygen 4.00 mg/L. The broodstocks were collected in July, 2019 and acclimatized for a month in 10 $m^2$ earthen pond in the FCFFT Hatchery complex. They were fed twice daily at 3% of their biomass with 40% protein diet.

### 1.2 Collection of pituitary gland

The pituitaries were collected from four (4) males as shown in Figure 1. The head of the fish was turned upside down, a sharp butchers knife was used to cut the lower jaw away (cranium). The skull was lifted up to expose the brain and the blood was cleaned off, showing the fatty lobe substances exposing the olfactory nerves of the brain. The pituitary gland was located on the top of the skull after gently removing the entire brain, it is a pinky white globule-like organs located on the ventral side of the brain. The pituitaries were collected with a pair of tweezers and put in a mortar and crushed, 2 mL of physiological solution was added (9 g of NaCl / 1 liter of distil water) (Shanthanagouda and Khairnar, 2018).

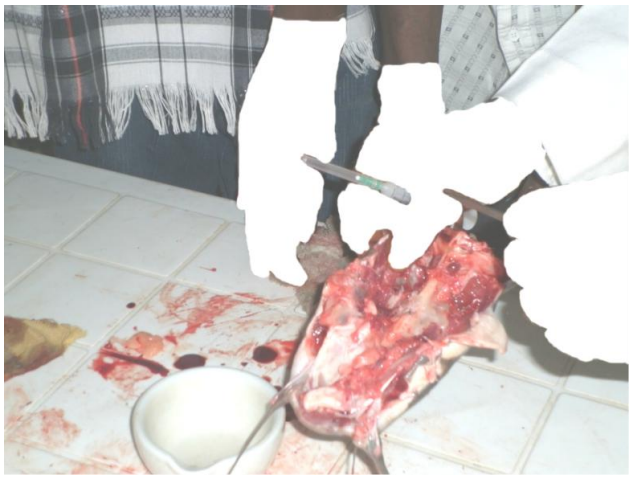

Figure 1 Extraction of pituitary gland

### 1.3 Hypophysation and induced breeding

The selected females and males were injected intramuscularly with ovaprim and pituitary gland extract in Figure 2. 0.5 mL/kg for ovaprim and 2 mL/kg of pituitary extract. The male received half of what was administered to the females. The injection of the two hormones were done at the same time, after which the injected breeders were kept in an aerated holding plastic tanks containing oxygenated water.

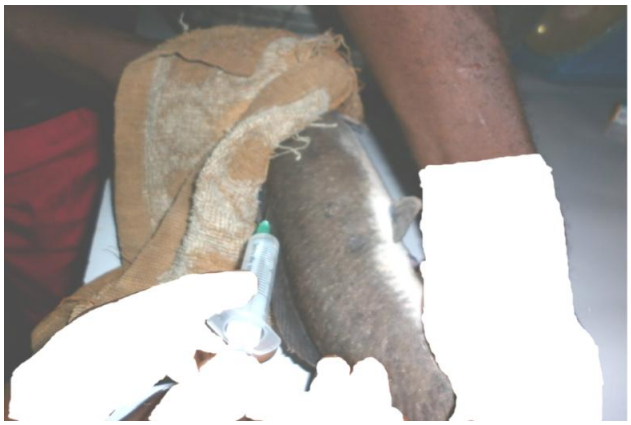

Figure 2 Injection of C. gariepinus with ovaprim/pituitary

### 1.4 Gametes collection

Hand stripping of females were carried out after the latency period of 10~12 hours respectively. The females were first cleaned with hand towel to avoid water having contact with the eggs during stripping. Stripping were done by applying pressure on the abdomen as shown in Figure 3, the fecundity was estimated. While for the testes the abdomen was gently dissected using surgical blade and the testis were located and measured using meter rule for the sperm length, 2 mL syringe was utilized to make an opening on the testis and the milt from each lobe was collected separately and measured Figure 4. The male was stitched as shown in Figure 5, and returned to spent tank for survival. During this period the stitched males remain for two weeks inside the water without feeding. The healed male was returned into the broodstock tanks and fed. Subsequently, it was ready for reuse after four (4) months respectively (Onyia et al., 2015).

Figure 3 Egg stripping

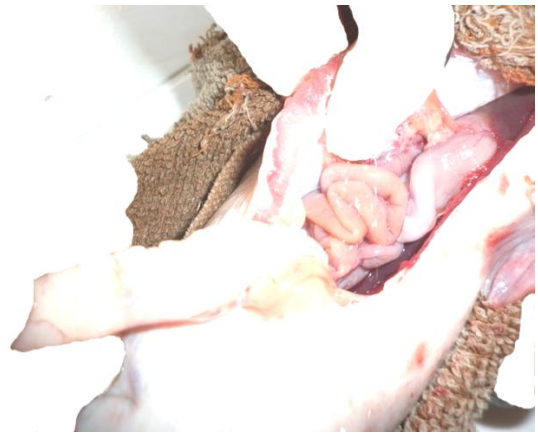

Figure 4 Collection of milt from the male broodstock

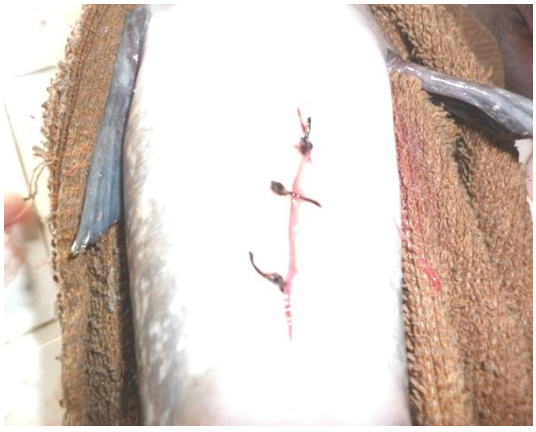

Figure 5 Stitched male (survived after two weeks)

### 1.5 Eggs fertilization and incubation

50 eggs from each treatment were placed in a dry Petri dish replicated five times, 0.5 mL of milt was added and allowed for 2 minutes to fertilize and counted under a microscope (mg x40). The fertilization rate was calculated as reported by (Ochokwu et al., 2016).

% Fertilization = Number of fertilized eggs/total number of eggs x 100
% Hatchability = Number of hatchlings/total number of incubated eggs x 100
% Survival = Number of fry /No. of stocked hatchlings x 100

### 1.6 Incubation of eggs (indoors)

However, 100 fertilized eggs from each treatment were replicated five times and incubated in well aerated rectangular concrete (indoor) tanks of 1 m x 1 m x 1 m in a complete randomized design arrangement to evaluate for the growth performance and survival. Hatchlings were fed ad-libitum with fish meal. After one week, pooled weight of fry in each tank were taking using a sensitive balance ACCULAB 333 to the nearest 0.1 g to adjust the feed, the growth in length were measured using meter rule (cm) and survival rate recorded as previously reported by Onyia et al. (2016).

### 1.7 Fingerling stage (outdoors)

At the end of the feeding trial for four weeks in an indoor facility, 50 fingerlings replicated five times were transferred to the nursery pond (2 x 2 x 1.5 m) in an outdoor pond and reared for four weeks in a complete randomized design setup. Fingerlings were fed with 40% crude protein diet (coppen feed) trice daily at 5% of their biomass. Initial pooled weight of the fingerlings/treatment were taken while weekly 20 fingerlings of each treatments were taken for length, weight measurements and survival were recorded for four (4) weeks in the concrete nursery ponds (outdoors).

### 1.8 Monitoring of physicochemical parameters

The following physicochemical parameters were monitored throughout the rearing period Temperature, Dissolve Oxygen, pH and conductivity.

### 1.9 Statistical analysis

Variations in the data generated from the two hormonal treatments were subjected to one – way- analysis of variance (AVOVA). The differences in the means that was significant at 5% were determined using Duncan multiple range test (DMRT) in an SPSS version 20. While the graphs were drawn using Excel software 2013.

## 2 Results

Table 1 revealed the fecundity, percentage fertilization/hatchability and survival of F1 reared for a week. *C. gariepinus* induced with ovaprim had the highest fecundity (10,500) while pituitary was 7,000. The percentage fertility and hatchability for fish induced with ovaprim was (71.4 and 70) respectively while the fish induced with pituitary gland had fertility (68.1) and hatchability (69). Survival rate after a week was highest in *C. gariepinus* induced with pituitary 65% and ovaprim had 62%.

Table 1 Mean Fertilization, hatchability and fecundity of *C. gariepinus* induced with Ovaprim and Pituitary gland

| Parameters/Treatments | Pituitary gland (2 mL/kg of fish) | Ovaprim (0.5 mL/kg of fish) |
|---|---|---|
| Weight of the female | 800 g | 800 g |
| Latency period/hour | 12 | 10 |
| Fecundity | 7,000$^{b}$ | 10,500$^{a}$ |
| Egg diameter before injection | 1 mm | 1 mm |
| Egg diameter after injection | 1.3 mm | 1.3 mm |
| Incubation period | 18-36 hours | 18 -36 hours |
| % Fertilization | 68.1$^{b}$ | 71.4$^{a}$ |
| % Hatchability | 69 | 70 |
| % Survival | 65$^{a}$ | 62$^{b}$ |

Note: Means with different superscript in same row are significantly different ($P$<0.05)

However, Table 2 uncover the sperm volume which was higher in the males induced with pituitary gland 3.2 mL, and also has the highest length of the testes 5.2, meanwhile the male induced with ovaprim had the highest weight of the testes 6.1 respectively.

Table 2 Mean sperm volume, weight of the testes and milt volume of *C. gariepinus* induced with ovaprim/pituitary gland

| Parameters/Treatments | Pituitary gland (mL/kg of fish) | Ovaprim (0.25 mL/kg of fish) |
|---|---|---|
| Weight of the male (g) | 1200 | 1200 |
| Weight of left testes (g) | 5.4[b] | 6.1[a] |
| Weight of right testes (g) | 5.7 | 5.3 |
| Length of left testes (cm) | 5.2 | 5.1 |
| Length of right testes (cm) | 4.8 | 4.2 |
| Milt volume (ml) | 3.2[a] | 2.8[b] |

Note: Means with different superscript in same row are significantly different ($P$<0.05)

Figure 6: represent the weekly mean weight relationship due to the effect of ovaprim and pituitary gland extract on production of clarias gariepinus fry reared indoors for four (4) weeks. There was no significant difference ($P$>0.05) in the final weight of fish induced with ovaprim after four weeks (6.22) and pituitary gland (6.82).

Figure 7: weekly increase in length of *C. gariepinus* fry induced with ovaprim/pituitary reared indoor. There was no significant difference across the treatments in length increase of the fish reared indoor. The final length for *C. gariepinus* hatchlings induced with pituitary was (6.82) and ovaprim (6.22).

Weekly weight (g) of *C. gariepinus* hatchlings induced with ovaprim/pituitary gland reared in outdoor pond is presented in Figure 8. The final weight of the fish induced with ovaprim after four weeks was 56.91 g and pituitary had 56.98 g, interestingly there was no significant difference among the treatments.

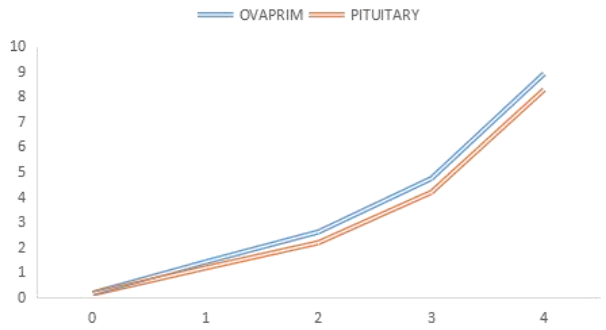


Figure 6 Weekly weight (g) of *C. gariepinus* induced with Ovaprim and Pituitary reared in indoor pond

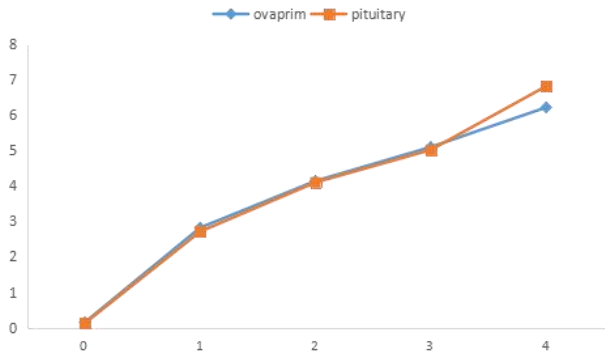


Figure 7 Weekly length (cm) of *C. gariepinus* induced with Ovaprim and Pituitary reared in an indoor pond

Figure 9 showed the increase in the length of the hatchlings reared in an outdoor pond. At the end of the four weeks of rearing, fish induced with ovaprim had 24.72 cm while pituitary had 24.69 respectively.

Figure 10: delineate the DO of the water used to rear the fry in an indoor pond. The DO recorded was 4.5 mg/L in pond water containing fish induced with pituitary and 4.2 in pond water treated with ovaprim.

While in Figure 11. Which represent the pond water used to rear the fingerlings in an outdoor pond. The DO was 3.4 mg/L for the pond treated with pituitary and 3.2 or the ovaprim treated ponds.

Figure 12 Shows the pH (7.2), conductivity (610) and Temperature (28$^{o}$C) of the fingerlings reared in an indoor pond. There was no significant difference.

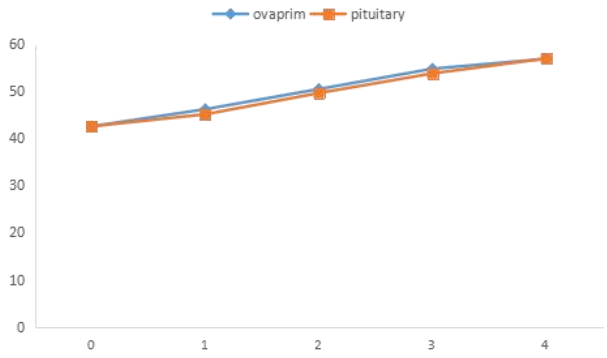


Figure 8 Weekly mean weight (g) of *C. gariepinus* induced with Ovaprim and Pituitary reared in outdoor pond

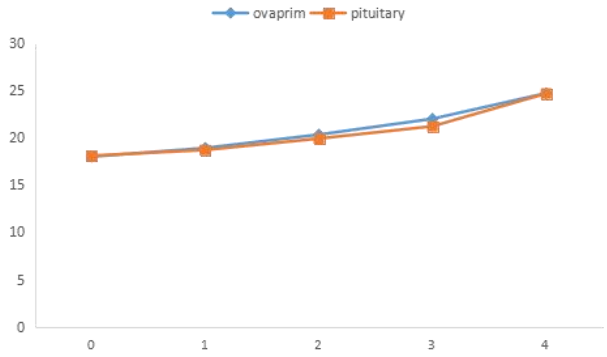


Figure 9 Weekly mean length (cm) of C. gariepinus induced with Ovaprim and Pituitary reared in outdoor pond

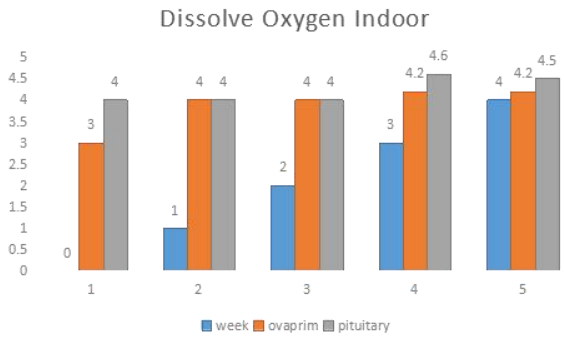


Figure 10 Dissolve oxygen level of the water used to rear the fry induced with Ovaprim and Pituitary reared in indoor pond

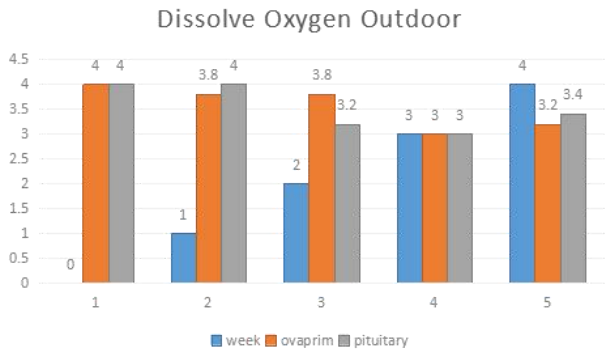


Figure 11 Dissolve oxygen level of the water used to rear the fry induced with Ovaprim and Pituitary reared in outdoor pond

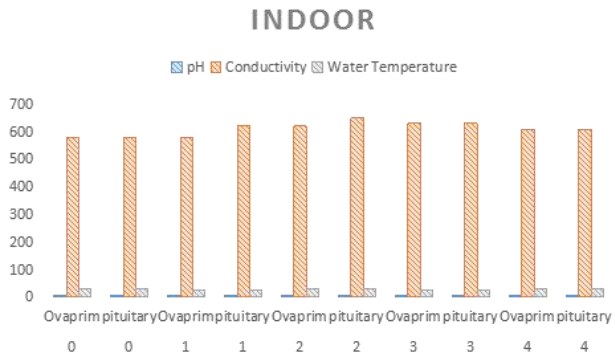


Figure 12 Some physicochemical parameters of the water used to rear the fry induced with Ovaprim and Pituitary reared in outdoor pond

Subsequently, in Figure 13. Same trend was observed in the water utilized to rear the fingerlings in an outdoor pond. pH 7.9 in pond treated with pituitary and 8.0 in pond treated with ovaprim, while the conductivity was 460 and temperature 30$^{o}$C.

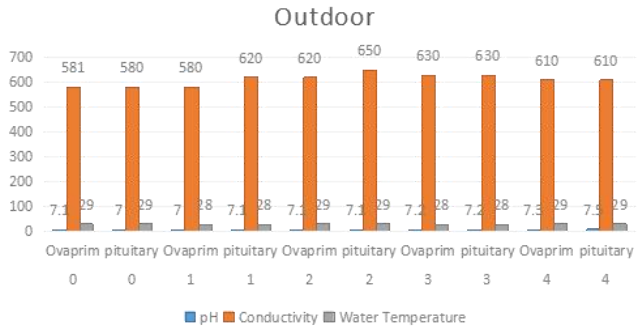


Figure 13 Some physicochemical parameters of the water used to rear the fry induced with Ovaprim and Pituitary reared in outdoor pond

Table 3 display the growth parameters of the fry reared in an indoor pond. There was no significant difference in the weight gain of the fry treated with pituitary (8.77) gland and ovaprim (8.13) also in mean length gain the fry induced with ovaprim had 6.08 and pituitary 6.7. However, the percentage survival was higher in pituitary induced *C. gariepinus* 78% and ovaprim 76%.

Table 4 indicate the growth parameters of the fingerlings reared in outdoor pond. There was no significant differences in the mean weight gain. Pituitary had 14.42 and ovaprim 14.35 respectively. The survival rate was higher in the fingerlings reared out door. Ovaprim had 98% while pituitary was 95% survival.

Table 3 Growth parameters of fry reared in an indoor pond

| Parameters | Ovaprim | Pituitary gland |
|---|---|---|
| Mean final weight (g) | 8.91 | 8.27 |
| Mean initial weight (g) | 0.14 | 0.142 |
| Mean weight gain (g) | 8.77 | 8.13 |
| Mean final length (cm) | 6.22 | 6.82 |
| Mean initial length (cm) | 0.143 | 0.117 |
| Mean length gain (cm) | 6.08 | 6.7 |
| % Survival | $76^{b}$ | $78^{a}$ |

Note: Means with different superscript in same row are significantly different ($P$<0.05)

Table 4 Growth parameters of the fingerlings reared in an outdoor pond

| Parameters | Ovaprim | Pituitary gland |
|---|---|---|
| Mean final weight (g) | 56.91 | 56.98 |
| Mean initial weight (g) | 42.56 | 42.56 |
| Mean weight gain (g) | 14.35 | 14.42 |
| Mean final length (cm) | 24.72 | 24.69 |
| Mean initial length (cm) | 18.0 | 18.1 |
| Mean length gain (cm) | 6.72 | 6.59 |
| % Survival | $98^{a}$ | $95^{b}$ |

Note: Means with different superscript in same row are significantly different (P<0.05)

## 3 Discussion

The result obtained in this research revealed the efficiency and profitability of induced breeding using natural hormone (*C.* gariepinus pituitary extract) and ovaprim. It exposed that there is no significant difference in using synthetic hormone (ovaprim) and natural hormone (pituitary gland) in inducing catfish breeding. In this research the weight of the broodstock used was males (1200 g) and females (800 g) and all responded positively to the hormones. Subsequently spawned 10 hours after injecting with hormones at 29$^{o}$C.

The fecundity rate was higher in the females induced ovaprim than females induced with pituitary extract, this coincide with Chattopadhyay (2018) who recorded increase in the fecundity of catfish (*Ompok pabda*) induced with ovaprim, meanwhile the fish injected with carp pituitary yet had high fecundity as also reported in this research, but do not comply with (Olaniyi and Akinbola, 2013) who reported increase in fecundity of fish induced with pituitary gland. However, Ganias (2018) defined fecundity as a measure of gamete produced which reveal the number of matured eggs released in a breeding period while Bradshaw and McMahon (2008) reported that fecundity is the physiological maximum potential reproductive output of a female fish within its lifetime and in totality typify one of the major fundamental of theoretical and applied population biology

Towers (2014) reported that the temperature, pH and season of the year could be a causative factors to increase or low in fecundity. Moreover, fish size and weight, egg diameter, viability of the eggs, latency period, and amount of toxin in the culture medium/water is inclusive.

The percentage fertilization and hatchability recorded in this research was higher in the fish induced with ovaprim. However, both treatments gave a high result to that effect. This agreed with (Hamid et al., 2005; Ndimele and Owodeinde, 2012; Olaniyi and Akinbola, 2013; Chattopadhyay, 2018); also Das et al. (2016) and Hossain et al. (2012) conveyed high fertility in fish induced with ovaprim but Hossain et al. (2012) also report high hatchability among the females induced with pituitary extract. Subsequently the fish induced with pituitary extract recorded high survival (65%) after one week of rearing in an indoor pond against the fish induced with ovaprim (62%), this concur with (Chattopadhyay, 2018).

The growth rate of the hatchlings induced with pituitary and ovaprim reared indoor and in an outdoor revealed that there was no significant difference (P<0.05) across the treatments. Ndimele and Owodeinde (2012) recorded poor growth rate across the treatments when compared with this research, moreover the highest weight gain (8.88 g)

observed in Ndimele and Owodeinde (2012) was in ovaprim induced fish reared for 56 days which was lower than the weight gain obtained in this research (14.35 g). Same trend was observed in Abdul et al. (2017), who recorded poor growth after feeding the fry for 28 days. Similarly Ikechukwu et al. (2019) recorded increase in weight of the fish induced with ovaprim and pituitary and further more stated that the fish induced with ovaprim had significant growth which did not differ from those induced with pituitary. This have proved that pituitary gland is an alternative for ovaprim which is costly not easily accessible in arid zone. The major causes of poor growth in fish farming is correlated with diet (feed, nutrients contents of the feed the fish consumed), the stocking rate, pH of the water, dissolve oxygen (Ochokwu et al., 2019). Another causative effect is the genetic makeup of the parent stock which is inherited by the offspring (Ochokwu et al., 2015). These traits have higher effects on the first generations.

Similarly, the percentage survival recorded in this work was high in both treatments and it concur with Ikechukwu et al. (2019) but disagreed with Ndimele and Owodeinde (2012) who recorded poor survival at the end of the research.

## 4 Conclusion

The research points out the essentiality of using pituitary gland from available *C. gariepinus* in the arid zone for breeding and effectively rearing the hatchlings for fingerlings availability in the zone. Both the synthetic hormone (ovaprim) and natural hormone (Pituitary extract from *C. gariepinus*) positively influenced the reproductive performance of the fish, it exerted positive change in fertility, hatchability growth in weight, length and survival of the hatchlings both in an indoor and outdoor rearing facility. Finally, the farmers who depend on capture fisheries for survival because of the poor access to synthetic hormone and cost of purchasing it can adopt the pituitary gland for induce breeding in the zone. However, in this research 2 mL/kg was used to inject the females and 1ml/kg was injected to the male, this can still change and should not be limited to only 2 mL/kg. The farmers should also be alert throughout the latency period to avoid under or over ripening of the eggs after injection.

### Authors' contributions

The research was divided into different phases in which the three authors listed, effectively contributed and positively to the completion of the research article. Dr. Ochokwu, I.J. Y.Y., Saidu, K.A, Technologist X.X., Prof. Bichi, A.H, Z.Z. conceptualization, X.X. and Y.Y.; methodology, Y.Y. and Z.Z; software, Y.Y. and X.X; validation, X.X., Y.Y. and Z.Z.; formal analysis, X.X. and Y.Y; investigation, X.X. Y.Y and Z.Z; resources, X.X. and Z.Z; data curation, X.X. and Y.Y; writing—original draft preparation, X.X. and Y.Y; writing—review and editing, X.X. and Y.Y and Z.Z; visualization, X.X.; supervision, X.X. and Y.Y; project administration, X.X.; funding acquisition, X.X. and Y.Y. All authors read and approved the final manuscript.

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